

The SNP (g.198655287 G>A) of AHSG gene polymorphism and its association with mineral composition in Indonesia lamb meat

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Abstract. The AHSG (Alpha2-Heremans-Schmid Glycoprotein) gene is indicated to have an essential role in mineral composition. This study aimed to identify the polymorphism of the AHSG gene and its association with the mineral composition of Indonesian lamb meat. The samples were 85 rams Indonesian sheep, consisting of 70 Javanese thin-tailed sheep (JTTS) and 15 Jonggol sheep (JS). AHSG gene polymorphism was analyzed using the PCR-RFLP technique with the EagI restriction enzyme. The AHSG gene association was analyzed using the T-test. The Hardy-Weinberg equilibrium analysis showed that the AHSG was polymorphic with three genotypes, i.e., GG, GA, and AA. The AHSG gene with mineral composition was associated with significance ($P < 0.05$) on the content of Fe (iron), Zn (Zinc), and Se (Selenium). The AA genotype is the recommended genotype due to its high mineral composition. The AHSG gene (SNP g.198655287 G>A) can be used as a genetic marker for the molecular-based selection of lamb with high mineral composition.

1 Introduction

Lamb meat is classified as a red meat that is preferred by consumers in each country, including Indonesia, due to its flavor, texture, and nutritional value [1]. Consumers have complex thoughts in assessing food products, especially meat, from several aspects, i.e., meat products have a tender, juicy texture, and quality flavor. Meat products also function as a source of protein and are nutritious, safe, and healthy food [2,3]. In addition to fatty acids and protein, meat provides an important dietary source for various other essential nutrients, including minerals needed by consumers. Genomic studies have explained that there is a relationship between mineral composition and muscle building, fat storage, and meat quality

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[4]. High mineral content is now a positive trend as a food additive. Sensory properties, including flavor, tenderness, and cheesiness of meat, have been associated with and influenced by several mineral contents [5]. Interactions between Potassium (K) and Iron (Fe) with cholesterol, Polyunsaturated Fatty Acid (PUFA), and Monounsaturated Fatty Acid (MUFA) in black Angus cattle reported by Pannier et al. [6].

Improving lamb mineral composition using genomic studies is an effective and efficient way to identify genes that control mineral composition. The gene that is indicated to have an association is the Alpha2-Heremans- Schmid Glycoprotein gene referred to as Fetuin-A, which is a glycoprotein and is encoded by the AHSG gene, which is synthesized and secreted by the liver [7]. AHSG is a glycoprotein functioning in bone minerals; AHSG can bind Ca^{2+} (calcium ions) with a broad scope to prevent the formation of apatite minerals in circulation and plays an essential role in bone cell metabolism in the human body [8]. The AHSG gene is positively associated with limousin beef quality on tenderness and cheese properties [9]. The AHSG gene is implicated in the regulation of body fat and insulin sensitivity, which are thought to influence body fat content and metabolism [10]. In transcriptomic and proteomic studies conducted on Hu and Dorper sheep longissimus thoracis, the AHSG gene is associated and up-regulated in lipid synthesis [11]. The AHSG gene is a gene controlling some fatty acid composition in some Indonesian sheep breeds that have low saturated and high unsaturated fatty acids at SNP (g. 198655287 G>A) [12]. It has also been reported that the AHSG gene circulates Fetuin-A, which correlates strongly with plasma cholesterol levels in humans [13]. Identification of the AHSG gene has never been done on meat mineral composition, primarily Indonesian sheep. This study aims to characterize the AHSG gene at SNP (g. 198655287 G>A) on mineral composition through PCR-RFLP.

2 Materials and methods

2.1 Experimental Samples

A total of 85 DNA samples were extracted from 70 Javanese Thin-Tailed Sheep (JTTS), and 15 Jonggol sheep (JS). The DNA samples were obtained from the longissimus dorsi muscle of the rams. Animals were reared under an intensive farming system with forage (Napier grass) and concentrated feed and water ad libitum. The lamb samples of 85 animals were stored at -20°C and analyzed for mineral composition. The animal ethics commission of IPB University has granted consent (approval number. 117-2018 IPB) for all procedures involving animals.

2.2 Mineral composition analysis

Meat was analyzed by the Atomic Absorption Spectrophotometry (AAS) method. The AAS method is an analytical method used to calculate the quantity of metal elements based on the absorption of radiation absorbance by free atoms in the gas phase. The mineral content of meat (longissimus dorsi) was analyzed in this study is potassium (K), iron (Fe), zinc (Zn), and selenium (Se). A total of 2.5 g of meat (longissimus dorsi) sample was put in an Erlenmeyer tube, and added 25 mL of concentrated HNO_3 and boiled for 30-35 minutes. The solution was cooled, and 10 mL of 70-72% HClO_4 was added. Then, the solution was slowly brought to a boil until the solution looked colorless. The solution was then cooled and added 50 mL of H_2O and boiled again until all NO_2 gas came out. The solution was then cooled and filtered into a 100 mL volumetric flask. The solution was then tested with an atomic absorption spectrophotometer (SSA) to obtain the mineral content of K, Fe, Zn, and Se in the meat.

2.3 DNA Extraction and Amplification

The longissimus dorsi samples were utilized for DNA extraction with the Geneaid gSYNC DNA extraction Kit protocol (Geneaid Ltd, Taiwan). The DNA extraction consisted of four stages, i.e., (1) Sample preparation, (2) Protein degradation, (3) Degradation of organic matter, and (4) DNA precipitation. After the extraction, the DNA was stored at -20 °C (Sambrook et al.1989). The SNP g.198655287 G>A and the design of the forward primer (5'-GGAGGAATCAGGGCATTTC-3') and reverse primer (5'CCCATATCCTTACGCAATCC-3') used for amplification refers to the research Munyaneza et al. [12] procedure with a product length of 473 bp.

The DNA was then amplified using the PCR technique, which has two stages, i.e., (1) Sample preparation: 2 µL of extracted DNA was distributed as much as into a 0.2 mL tube, and prepared 14 µL of solution mixture of 6.1 µL NFW (Nuclease Free Water), 0.2 µL forward primer, 0.2 µL reverse primer, 7.5 µL My Taq HS Red Mix, then mixed using a mini centrifuge. (2) Samples incubation: The mixture is inserted in a thermocycler machine (ESCO Scientific, Singapore) for DNA amplification. The machine's operation commences with a 5-minute denaturation at 95 °C. The subsequent step involves 35 cycles of denaturation at 95 °C for 10 seconds, primary annealing at 58 °C for 20 seconds, and DNA extension at 72 °C for 30 seconds. Final extension is primer elongation at 72 °C for 5 minutes. The results of DNA amplification were visualized using electrophoresis (1.5% agarose gel).

2.4 Genotyping using PCR-RFLP technique

The genotyping procedure used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique with the restriction enzyme EagI. The samples were incubated at a temperature of 37 °C for 4 hours (Thermo Fisher Scientific, EU, Lithuania). The DNA then were distributed as much as 5 µL into a 0.5 mL tube and mixed with 2 µL of a mixture consisting of 0.9 µL NFW (Nuclease Free Water), 0.7 µL buffer, and 0.4 µL EagI restriction enzyme. After incubation, the samples were visualized by electrophoresis technique (1.5% agarose gel). The genotypes marker of the AHSG gene were AA: 473 bp; GA: 473, 273, 200; and GG: 273, 200.

2.5 Data Analysis

The gene polymorphism was identified using Pop Gen 32 software to calculate the genotype frequency, allele frequency, and Hardy-Weinberg equilibrium. The AHSG gene association with the mineral composition was identified using the T-test statistical model of the SAS 9.2 software. The Allele frequency and genotype frequency were calculated based on the following procedure Hartl and Clark [14]. The Hardy-Weinberg equilibrium was calculated with the following approach Hartl and Clark [14]. The association of the AHSG gene genotypes with mineral content was described using the T-test [15]:

$$t = \frac{(X_1 - X_2)}{\delta^2 \frac{\sqrt{1}}{n_1} + \delta^2 \frac{\sqrt{1}}{n_2}} \delta^2$$

Note :

X1 and X2 = mean mineral composition of genotypes 1 and 2

n1 and n2 = number of individuals of genotypes 1 and 2

δ2 = combined variance

A normality test was conducted before a T-test using the Kolmogorov-Smirnov or Shapiro-Wilk test. If the data were abnormal, then the outlier data is removed. If all outliers data were already removed and the data was still abnormal, then the data were transformed before analyzing the data using the T-test [16].

3 Result and Discussion

3.1 AHSG gene polymorphism in the Indonesia sheep population

AHSG gene SNP (g.198655287 G>A) polymorphism was performed using PCR-RFLP technique with an EagI restriction enzyme and obtained three fragment products, i.e. GG, GA, and AA. Fragment product results were visualized using an electrophoresis technique, which can be seen in Figure 1. In this case, the restriction enzyme selection recognized and cut the target DNA sequence [17], resulting in different fragment products [18]. The mutated base of the AHSG|EagI gene is A instead of G. The fragment product produced by the GG genotype contains two fragments with lengths of 273 bp and 200 bp, while the fragment product produced by the GA genotype contains three fragments with lengths of 473 bp, 273 bp, and 200 bp, and the genotype has one fragment with a length of 473 bp.

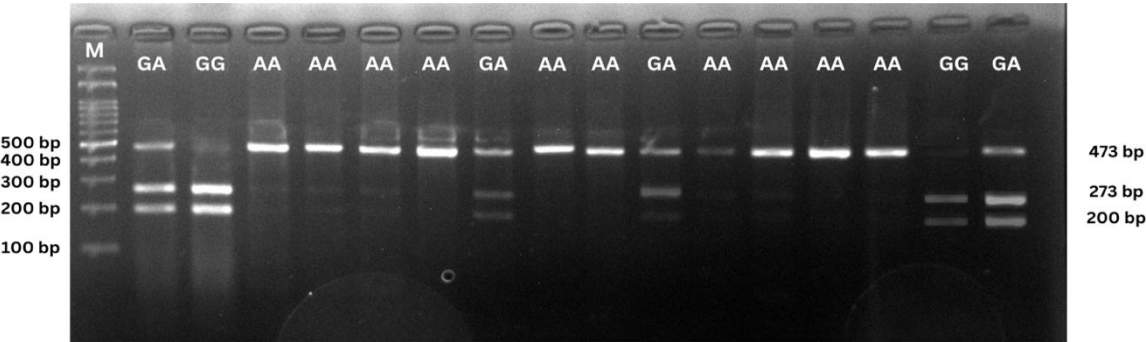


Fig. 1. Visualization results of AHSG gene (g.198655287 G>A) in Indonesian sheep with three genotypes: GG, GA, and AA using 2% agarose gel and 100 bp marker.

The AHSG|EagI gene polymorphism in Indonesian sheep produces GG, GA, and AA genotypes. The GA genotype was identified as the most dominant with a frequency of 0.46, followed by the AA genotype at 0.41 and the GG genotype at 0.13. This result indicates that the A allele is the most common compared to the G allele in the sheep population studied. The AHSG|EagI gene is categorized as a polymorphic because the overall genotype frequency of the sheep used less than 0.99, including the JTTS, JS, and total population.

The SNP in the gene are categorized as polymorphic if they have more than one allele with a frequency value of at least 1% [19,20]. The results of the chi-square analysis showed significant differences, indicating that the AHSG|EagI gene in the Indonesian sheep and Javanese thin-tailed sheep (JTT), Jonggol sheep (JS) and total population is the allele is not in equilibrium, which indicates the population may have experienced migration, mutation, selected mating, or selection [21].

Table 1. Genotype and allele frequencies and Hardy-Weinberg equilibrium of AHSG gene in Indonesian sheep population

No	Breed	n	Genotype frequency			Allele frequency		Chi-square (X ²)
			GG	GA	AA	G	A	
1	Javanese thin-tailed sheep (JTTS)	70	0.13 (4)	0.46 (42)	0.41 (24)	0.36	0.64	6.58
2	Jonggol Sheep (JS)	15	0.37 (4)	0.63 (11)	0.00 (0)	0.37	0.63	5.03
3	Total population	85	0.13 (4)	0.46 (53)	0.41 (28)	0.36	0.64	10.71

n = number of samples;
 χ^2 table= 3.84

3.2 AHSG gene with mineral composition

The AHSG gene SNP (g.198655287 G>A) has a significant association with mineral composition, including Ferrum (Fe), Zinc (Zn), and Selenium (S). The results of mineral composition in Indonesian sheep on the composition of Fe, Zn, and Se have values of 1.58-2.04 mg/100g, 2.42-3.05 mg/100g, and 0.81-0.89 mg/100g, respectively. The results of mineral composition in Indonesian sheep on the composition of Fe, Zn, and Se each have a value of 1.58-2.04 mg/100g, 2.42-3.05 mg/100g, and 0.81-0.89. mineral composition in Indonesian sheep has similarities to the minerals contained in three Croatian [22] and Marocco [23] indigenous sheep breeds, especially in Fe and Zn.

Table 2. Association of AHSG Gene with mineral composition

Mineral	GG ($\bar{x} \pm sd$) (n)	GA ($\bar{x} \pm sd$) (n)	AA ($\bar{x} \pm sd$) (n)	P value		
				GG vs GA	GG vs AA	GA vs AA
Fe	1.58 ± 0.3 (4)	1.71 ± 5.02 (50)	2.04 ± 0.44 (25)	ns	*	**
Zn	2.42 ± 0.48 (4)	2.51 ± 9.93 (53)	3.05 ± 1.01 (28)	ns	ns	**
K	268.85 ± 52.51 (4)	281.83 ± 92.68 (53)	256.45 ± 83.00 (28)	ns	ns	ns
Se	0.89 ± 0.06 (2)	0.73 ± 0.13 (46)	0.81 ± 0.06 (20)	ns	ns	*

$\bar{x}$: mean (mg/100 g)
 Sd : standard deviation
 P value : significancy between genotype
 ** : significant different at P<0.01
 * : significant different at P<0.05
 ns : not significant

Genotype AA on the composition of Fe, Zn, and Se is very significantly different compared to genotype GA (P <0.01), and especially on the composition of Fe also has a significant effect compared to genotype GG. Genotype AA has the highest value if with genotype GA and GG, especially on the composition of Fe and Zn. Fe and Zn composition in meat (leanness) is strongly influenced by muscle fiber type and muscle oxidative capacity [22]. Fe and Zn also have a positive effect on lipid metabolism [4]. Transcriptomic studies explain that the AHSG gene affects lipid synthesis (up-regulated) [11] and is indicated to be positively associated with Fe and Zn composition. The advantage of high Zn composition in lamb meat is that the body can absorb it well [24]. The influence on parameters with yield can be due to several factors, including breed, age, and muscle [23]. However, the

composition of selenium is strongly influenced by feeding patterns and geographical location; case studies explain that sheep in pasture are rich in selenium composition [25] and Se composition in Indonesian sheep are higher in value when compared to Croatian [22] and Marroco [23] indigenous breeds sheep.

4 Conclusion

The AHSG gene SNP (g.198655287 G>A) in Indonesian sheep, showed polymorphic with three genotypes, i.e., GG, GA, and AA, and the distribution alleles were not in the Hardy Weinberg equilibrium. In addition, the AHSG gene was significantly associated with Ferrum (Fe), Zinc (Zn), and Selenium (Se). The AA genotype could be a candidate marker for producing lamb with high mineral composition.

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